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The Epithelial Ca^{2+} Channel TRPV5 in Health and Disease

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The recent identification of the epithelial Ca^{2+} channel, TRPV5, in kidney represents a major step forward in our knowledge of renal Ca^{2+} handling. This membrane channel protein is the first member of a new family of Ca^{2+}-selective cation channels. It consists of 6 transmembrane spanning domains, including a pore forming hydrophobic stretch between domain 5 and 6. TRPV5 constitutes the apical entry mechanism of active, transcellular Ca^{2+} reabsorption. In contrast to the paracellular route, this transcellular pathway enables the organism to actively control the net amount of Ca^{2+} reabsorption. *In vivo* studies indicated a specific regulation of TRPV5 by calcitriol, oestrogens and dietary Ca^{2+}. The central role of TRPV5 in active Ca^{2+} reabsorption makes it a prime target for pharmacological manipulation and several disorders related to Ca^{2+} homeostasis could benefit from such developments. This review highlights the identification, characteristics and the clinical impact of the epithelial calcium channel, TRPV5.

Key words: active Ca^{2+} transport, channel (in)activation, kidney, oestrogens, trafficking, TRP regulation, vitamin D

IMPORTANCE OF CA^{2+} HOMEOSTASIS

The maintenance of the Ca^{2+} balance within the physiological range is pivotal for life. Ca^{2+} is the most abundant cation in the human body where it is essential for many physiological functions, such as synaptic transmission in neurons, muscle contraction, blood clotting, fertilization and bone mineralization. The extracellular Ca^{2+}

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At the cellular level, active Ca^{2+} reabsorption is generally envisaged as a 3-step process (Fig. 1B) consisting of passive entry of Ca^{2+} across the luminal or apical membrane, cytosolic diffusion of Ca^{2+} bound to vitamin D_3-sensitive Ca^{2+}-binding proteins (calbindin-D_{28K} and/or calbindin-

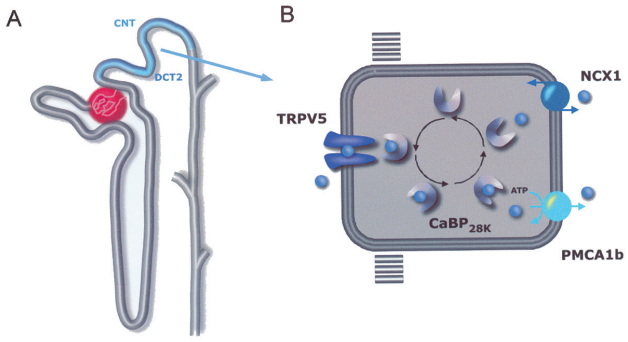

Fig. 1 (A) Model of the nephron, the functional unit of the kidney, depicting the TRPV5 expressing sections in the distal convoluted tubules (DCT) and connecting tubules (CNT). (B) Cell model of transcellular Ca^{2+} transport. Ca^{2+} enters the apical side of the epithelial cell through the TRPV5 channel, binds to the cytosolic calbindin-D_{28K} ($CaBP_{28K}$) and is extruded at the basolateral side via the plasma membrane Na^+-Ca^{2+}-exchanger (NCX1) and/or Ca^{2+}-ATPase (PMCA1b).

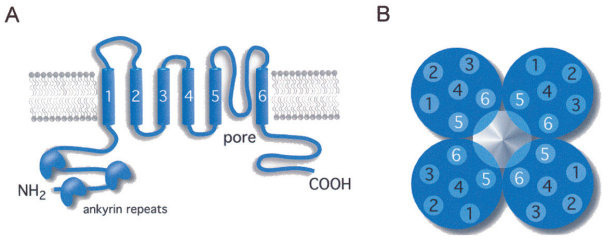

Fig. 2 (A) Schematic representation of a single TRPV5 subunit spanning 6 times the plasma membrane with a short hydrophobic stretch between transmembrane segments 5 and 6 that forms the pore of the channel. (B) Model of the molecular assembly of four TRPV5 proteins in the homotetrameric ring-like structure around the pore of the channel.

D_{9K}) and active extrusion of Ca^{2+} across the opposite basolateral membrane by the Na^+-Ca^{2+}-exchanger (NCX1) and/or Ca^{2+}-ATPase (PMCA1b)[8]. Apical Ca^{2+} influx is the rate-limiting step of the whole process and is, therefore, the most efficient target for hormonal regulation. The molecular identity of the apical Ca^{2+} entry pathway remained elusive until the identification of the epithelial Ca^{2+} channels TRPV5 (previously named ECaC1)[9] and TRPV6 (previously named Ca^{2+} transporter 1)[10,11]. TRPV5 and TRPV6 constitute a distinct class of highly Ca^{2+}-selective channels within the superfamily of transient receptor potential (TRP) channels, which encompasses a diversity of non-voltage cation channels[12,13]. The tissue distribution of both channels has been studied extensively by Northern blot, RT-PCR analysis and immunohisto-chemistry[7,14-17]. In humans both channels are coexpressed in the organs that mediate transcellular Ca^{2+} transport, including duodenum, jejunum, bone and kidney. This review will focus primarily on TRPV5, because this Ca^{2+} channel is the major isoform in the kidney.

MOLECULAR FEATURES OF TRPV5

The TRPV5 gene is located on human chromosome 7q35 and comprises 15 exons encoding a protein of about 730 amino acids[18]. This functional channel complex is a tetramer[19] where 4 subunits presumably form a ring-like structure around a central pore (Fig. 2A). Each subunit spans the apical plasma membrane 6 times and is predicted to form a pore region between transmembrane segments 5 and 6[6] (Fig. 2B). TRPV5 exhibits high Ca^{2+} selectivity and permeation over Na^+ due to a single aspartate residue (D542) present in the putative pore-forming region of each subunit[20]. Furthermore, TRPV5 is an inwardly rectifying channel and exhibits a Ca^{2+}-dependent feedback mechanism regulating channel activity[21,22].

REGULATION OF TRPV5 ACTIVITY

A tight control of TRPV5 activity is of primordial importance for the survival of the epithelial cells expressing the channel, as well as for the final concentration of reabsorbed Ca^{2+} in the body. Thus, various regulatory mechanisms exist which act on different levels: transcription, translation, trafficking to the plasma membrane and direct (in)activation of the apical channels (Fig. 3).

Transcriptional Regulation

TRPV5 is subjected to "long-term" hormonal regulation of transcription that occurs in the nucleus of the cell. 1,25-dihydroxy-vitamin D_3 (1,25-$(OH)_2D_3$), dietary Ca^{2+} and 17β-oestradiol are the main regulators acting on gene transcription of the channel[23-25].

Vitamin D is one of the most important regulators of the body Ca^{2+} balance and is required for proper development and maintenance of bone mass[26]. Previous studies reported

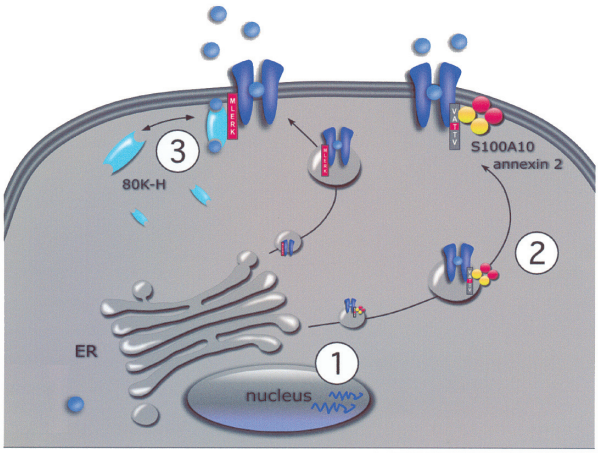

Fig. 3 Integrated model depicting the three different points of TRPV5 regulation: (1) transcriptional regulation by vitamin D_3 ($1,25\text{-}(OH)_2D_3$), dietary Ca^{2+} and oestrogens (17β-oestradiol), (2) regulation of TRPV5 routing to the plasma membrane by the S100A10-annexin 2 complex and (3) direct (in)activation of TRPV5 channel at the plasma membrane by the 80K-H Ca^{2+} sensor.

a stimulatory effect of vitamin D, via its active metabolite, $1,25\text{-}(OH)_2D_3$, on Ca^{2+} reabsorption[27,28]. Given the importance of the TRPV5 channel in active Ca^{2+} transport, the regulation of TRPV5 by $1,25\text{-}(OH)_2D_3$ has been investigated using two different models of vitamin D deficiency: mice lacking the $1,25\text{-}(OH)_2D_3$ receptor (VDR) or mice presenting decreased $1,25\text{-}(OH)_2D_3$ serum levels due to the ablation of 25-hydroxyvitamin D3-1α-hydroxylase (1α-OHase) which catalyses the synthesis of $1,25\text{-}(OH)_2D_3$. In both cases TRPV5 expression levels were significantly decreased compared to wild-type littermates[23,25,29]. Repletion of these animals with $1,25\text{-}(OH)_2D_3$ and/or dietary Ca^{2+} restored the hypocalcemia in the 1α-OHase knockout mice, which is accompanied by normalization of TRPV5 mRNA expression levels. Similar results were shown for vitamin D-depleted hypocalcemic rats in which TRPV5 was hardly detectable[17], suggesting that the transcription of TRPV5 is indeed controlled by $1,25\text{-}(OH)_2D_3$. This is strengthened by the elucidation of the human and murine TRPV5 promoter that contains four putative vitamin D-responsive elements (VDRE)[16-18]. Presumably, $1,25\text{-}(OH)_2D_3$ directly activates the promoter of TRPV5. Furthermore, the stimulatory effect of TRPV5 protein level upon $1,25\text{-}(OH)_2D_3$ administration to vitamin D-depleted animals suggests a possible (post)-translational regulation of TRPV5 by $1,25\text{-}(OH)_2D_3$[17,23].

Several of the aforementioned studies have provided evidence that $1,25\text{-}(OH)_2D_3$ regulates TRPV5 channel expression. It is, however, difficult to distinguish the effects of hypocalcemia from those of $1,25\text{-}(OH)_2D_3$ deficiency. Vitamin D-depleted hypocalcemic rats[17] and mice[23] are rescued not only by repletion of $1,25\text{-}(OH)_2D_3$, but also by dietary Ca^{2+} supplementation. Interestingly, dietary Ca^{2+} enrichment resulted in the normalization of the reduced TRPV5 expression levels in the 1α-OHase knock-out mice, as well as for the other Ca^{2+} transport proteins participating in active reabsorption[23]. Thus, induction of TRPV5 transcription by Ca^{2+} might involve activation of a putative Ca^{2+}-responsive element. Several domains have been proposed to function as Ca^{2+}-sensitive transcriptional regulators, including the serum-responsive element and the cAMP/Ca^{2+}-responsive element[30]. However, the presence and functionality of putative vitamin D and Ca^{2+}-responsive elements in the TRPV5 promoter remain to be investigated.

Oestrogen participates also in the transcriptional regulation of TRPV5. It is known that oestrogen is involved in bone mineralization[31] and that the deleterious effects of oestrogen deficiency after menopause leads to a negative Ca^{2+} balance associated with postmenopausal osteoporosis[32]. In ovariectomized 1α-OHase knockout mice, 17β-oestradiol supplementation resulted in elevated renal TRPV5 mRNA and protein levels, accompanied by normalization of the plasma Ca^{2+} levels[24]. Thus, TRPV5 transcription is controlled by oestrogen independently of vitamin D. These data suggested that the function of oestrogen in maintenance of the Ca^{2+} balance might be at least in part fulfilled by the regulation of TRPV5, thereby controlling (re)absorption of the amount of Ca^{2+} that is needed for maintaining the calcium balance. The mechanism of oestrogen-controlled up-regulation of TRPV5 mRNA remains to be elucidated, because oestrogen-responsive elements were not found in the putative TRPV5 promoter region[16].

Regulation of Trafficking to the Plasma Membrane

Modulation of the TRPV5 activity also occurs through translocation of the channel from intracellular pools to the plasma membrane. However, research on TRPV5 routing from the endoplasmic reticulum/Golgi compartment to the plasma membrane is still in its infancy. Immunohistochemical studies revealed that besides the apical TRPV5 localization, there is significant intracellular staining. In the latter compartment, TRPV5 could act as an intracellular reservoir of channels in order to be inserted in the plasma membrane when Ca^{2+} is needed[7,17]. In contrast to the "long-term" transcriptional regulation, trafficking of TRPV5 channels to the plasma membrane could provide a

"short-term" regulatory mechanism to increase Ca^{2+} reabsorption. It would be interesting to elucidate the molecular mechanisms including accessory proteins that play a role in the trafficking process of TRPV5. To this end, we recently demonstrated a regulatory role for the S100A10-annexin 2 complex in TRPV5 routing[33]. The S100A10-annexin 2 complex plays an important role in biological processes including endocytosis, exocytosis and membrane-cytoskeletal interactions[34]. Our studies showed that annexin 2 forms a well-defined heterotetrameric complex with S100A10 and associates with the TRPV5 carboxyl-terminal tail. Disruption of the S100A10-binding motif in TRPV5 resulted in abolishment of channel activity. This effect was accompanied by a major disturbance in the subcellular localization of TRPV5. Furthermore, down-regulation of annexin 2 using annexin 2-specific small interference RNA (siRNA) significantly inhibited TRPV5-mediated currents. Together these results demonstrated that the S100A10-annexin 2 complex is an important component for the trafficking of TRPV5 and TRPV6 to the plasma membrane.

Direct (In)Activation

TRPV5 is constitutively active, unlike many other TRP channels that are activated upon binding of ligands. This implies that in order to regulate TRPV5 activity, "short-term" acting mechanisms must exist to control the activity of the channels located at the plasma membrane to have a fast response based on a physiological stimulus. Importantly the amino- and carboxyl-terminal tails of TRPV5 contain potential regulatory motifs, such as, ankyrin repeats, PDZ motifs, and protein kinase C (PKC) phosphorylation sites[8].

The ankyrin repeat is a common protein sequence motif present in a large family of membrane-associated proteins that connect via their membrane-binding domains to diverse proteins, including proteins involved in Ca^{2+} homeostasis, such as inositol triphosphate (IP3) and ryanodine receptors[35,36]. In addition, recent studies on the TRPV5 homologue TRPV6 showed that ankyrin repeats are required for physical assembly of functional tetrameric channels[37]. Detailed molecular studies are now feasible and necessary to delineate the function of TRPV5 ankyrin motifs.

PDZ motifs are recognized by PDZ domains that are modular protein interaction domains. They can facilitate biological processes including linkage of ion channels to the cytoskeleton, as well as targeting of ion channels in correct spatial arrangement in relation to each other and specialized regions of the cell[38-40]. Several PDZ domains have recently been identified in renal proteins that could interact with apical transporters such as Na^+-H^+ exchanger (NHE), renal outer medullary potassium (ROMK) channel, cystic fibrosis transmembrane regulator (CFTR), the Na^+-phosphate (NaPi) transporter and TRPV5[41-44]. It was demonstrated that TRPV5 is the target of a complex regulating mechanism involving the PDZ motif protein NHE regulating factor 2 (NHERF2) and the serine/threonine kinases SGK1 and 3. The concerted action of NHERF2 and the kinases mentioned above markedly up-regulated the activity of TRPV5 channel[45].

Previous studies indicated an important regulatory role of PKC isoforms in hormonal regulation of transcellular Ca^{2+} reabsorption[4,6]. Theoretically, PKC could phosphorylate TRPV5 directly or indirectly via other PKC substrates. Despite the fact that TRPV5 contains several conserved PKC phosphorylation sites, present in the carboxyl-terminus, there is no experimental data showing direct phosphorylation of the channel. Recently, we demonstrated a role for the PKC substrate (80K-H) in the Ca^{2+}-dependent regulation of TRPV5 channel activity. Our study showed that 80K-H and TRPV5 co-localize in 1,25-$(OH)_2D_3$-responsive epithelia where they form a heteromeric complex along the plasma membrane[46]. 80K-H bound to the carboxyl-terminal tail of TRPV5 and controlled TRPV5 activity in a Ca^{2+}-dependent manner via its two EF-hand structures. Electrophysiological studies using 80K-H mutants showed that three domains of 80K-H (the two EF-hand structures, the highly acidic glutamic stretch and the HDEL sequence) are critical determinants of TRPV5 activity. Importantly, inactivation of the EF-hand pair reduced the TRPV5-mediated Ca^{2+} current and increased the TRPV5 sensitivity to intracellular Ca^{2+}, accelerating the feedback inhibition of the channel. None of the 80K-H mutants altered the TRPV5 plasma membrane localization or the association of 80K-H with TRPV5, suggesting that 80K-H has a direct effect on TRPV5 activity[46]. 80K-H is the only characterized protein so far that can act as Ca^{2+} sensor regulating the epithelial Ca^{2+} channel TRPV5.

PHENOTYPE OF THE TRPV5 KNOCK-OUT MICE

As described in the aforementioned paragraphs, TRPV5 has the critical determinants for active Ca^{2+} transport across the renal epithelia. In order to understand the function of this channel *in vivo* Hoenderop et al. realized genetic ablation of TRPV5 in mice, allowing further investigation of the physiological function of the Ca^{2+} channel[19]. Balance studies demonstrated that mice lacking TRPV5

(TRPV5$^{-/-}$) excreted six times more Ca^{2+} in their urine compared to wild-type mice (TRPV5$^{+/+}$). Besides this significant calciuresis, polyuria and polydipsia was consistently observed in TRPV5$^{-/-}$ mice when compared to control littermates. The large quantity of urine excretion in TRPV5$^{-/-}$ mice reduced the potential risk of Ca^{2+} precipitations, which could be formed due to the high urinary Ca^{2+} concentration. The hypercalciuria-induced polyuria has been observed previously in humans and animal models[47,48]. The acidification of urine that occurred in TRPV5$^{-/-}$ mice, contributed to the prevention of stone formation, since the formation of Ca^{2+} precipitates is less likely at an acidic pH[49]. To locate the defective site of the Ca^{2+} reabsorption along the nephron *in vivo* micropuncture studies were subsequently performed. Collections of tubular fluid revealed normal Ca^{2+} reabsorption in TRPV5$^{-/-}$ mice up to the last surface loop of the late proximal tubule. In contrast, the Ca^{2+} delivery to puncturing sites within DCT and CNT was significantly enhanced in TRPV5$^{-/-}$ mice. It is worthwhile to note that TRPV5$^{-/-}$ mice exhibited a significant increase in the rate of the intestinal Ca^{2+} absorption indicating a compensatory role to maintain a normal Ca^{2+} balance. Finally, TRPV5$^{-/-}$ mice exhibited a significant disturbance of the bone structure, including reduced trabecular and cortical bone thickness. Together, these data demonstrated that the lack of TRPV5 results in important disturbances not only of Ca^{2+} (re)absorption, but also of the whole body Ca^{2+} homeostasis.

TRPV5 IN RELATION TO HUMAN DISEASES

The role of TRPV5 in diverse Ca^{2+}-related disorders has been considered including variation in the urinary Ca^{2+} excretion during treatment with pharmaceutical agents, nephrolithiasis, idiopathic hypercalciuria (IH) and postmenopausal osteoporosis.

Thiazide diuretics, widely used in hypertension therapy, have the unique characteristic of increasing renal Na^+ excretion, while decreasing Ca^{2+} excretion[50]. These diuretic agents decrease renal Na^+ reabsorption by inhibiting the apical Na^+-Cl^- cotransporter (NCC) in DCT, resulting in an increased salt and water loss, and thereby decrease the extracellular volume (ECV)[51]. The decreased Ca^{2+} excretion during chronic thiazide administration has been explained by an increased passive Ca^{2+} transport in proximal tubules as well as a direct stimulation of active Ca^{2+} reabsorption in the DCT[7,52,53]. This hypocalciuric effect could provide therapeutic opportunities in IH and nephrolithiasis. Costanzo et al. proposed a mechanism where acute administration of chlorothiazide in the tubular lumen stimulates transcellular Ca^{2+} transport in DCT[50]. However, later studies suggest that the ECV contraction occurring during chronic thiazide treatment is primarily responsible for the hypocalciuria by enhancing the paracellular Ca^{2+} reabsorption[54-56]. In addition, thiazides decreased the mRNA expression and protein abundance of several transporters responsible for active Ca^{2+} reabsorption, regardless of volume status or calciuresis[57], excluding thus a stimulatory role of TRPV5 in chronic thiazide-induced hypercalciuria.

The hypercalciuria induced by the treatment with the immunosupressant drug tacrolimus has been attributed to decreased renal Ca^{2+} reabsorption and increased bone resorption[58-60]. A recent study showed that administration of tacrolimus for 7 days not only induces an increase of renal Ca^{2+} excretion, but also down regulates the renal mRNA expression of proteins involved in active Ca^{2+} transport including the TRPV5 channel[61]. These results together with the fact that serum Ca^{2+} concentration was unaltered support the hypothesis that tacrolimus induces a primary defect in renal active Ca^{2+} reabsorption by specifically inhibiting the transcription of Ca^{2+} transport proteins.

When hypercalciuria is combined with normal serum Ca^{2+} levels in the absence of any known underlying cause, it is idiopathic and termed IH. The pathogenesis of this autosomal dominant disorder is either excessive intestinal Ca^{2+} absorption (absorptive IH) or defective renal tubular Ca^{2+} reabsorption[62], which are both in line with the normocalcemic and hypercalciuric phenotype of the TRPV5 knock-out mice described above. Linkage analysis was effectuated for 9 families and a phenotype suggesting a primary renal defect. There were, however, no mutations identified in the open reading frame containing 15 exons and 3 kb of the 5'-flanking region of the TRPV5 gene[63]. The involvement of TRPV5 gene in IH cannot be completely excluded because the IH population is a heterogeneous group. Moreover, activating or silencing mutations in TRPV5 can hypothetically lead to primary renal as well as absorptive IH. In addition, single nucleotide polymorphisms (SNPs) in the Ca^{2+}-sensing receptor (CaSR) have been shown to significantly increase the relative risk of hypercalciuria and, therefore, may be involved in the complex genetic background of IH[64]. The same may be applicable to SNPs in the gene encoding the epithelial Ca^{2+} channel or genes involved in the regulation of TRPV5 activity.

It has been previously demonstrated that oestrogen deficiency after menopause is associated with increased renal Ca^{2+} loss[65]. This perturbation can be corrected by oestrogen replacement therapy and is not attributable to an

Table 1 Molecular targets of TRPV5 regulation

Regulation	Molecular target	Effect	Related disease	Reference
Transcription (1)	TRPV5 promotor	Abundance of TRPV5 mRNA	Hypercalciuria, postmenopausal osteoporosis and nephrolithiasis	[16-18], [24], [61], [63]
Routing (2)	S100A10	Density of TRPV5 on the plasma membrane		[33]
Direct (in)activation (3)	80K-H	Control of cellular Ca^{2+} influx		[46]

(1), (2), (3) correspond to numbers in figure 3.

increased filtered load, thereby suggesting a direct effect on renal Ca^{2+} reabsorption[66]. Furthermore, kidney stones develop less commonly in premenopausal women than in men. This has been associated with a lower urinary Ca^{2+} excretion relative to age-matched males, suggesting that oestrogen protects against Ca^{2+} nephrolithiasis via an increased reabsorption of Ca^{2+} [67-69]. Van Abel et al. studied the effect of oestrogen on the proteins involved in active Ca^{2+} reabsorption and showed that the presence of oestrogen may protect premenopausal women against Ca^{2+} nephrolithiasis by increasing TRPV5 expression and stimulating Ca^{2+} reabsorption[24].

CONCLUSIONS

The epithelial Ca^{2+} channel TRPV5 plays an essential role in active Ca^{2+} reabsorption and, therefore, in Ca^{2+} homeostasis. Active transcellular Ca^{2+} transport involves a chain of Ca^{2+} transport proteins facilitating the apical influx of Ca^{2+}, transport to the basolateral membrane and extrusion into the blood stream. Regulation of the TRPV5 channels that mediate the rate-limiting Ca^{2+} entry step is pivotal to control the Ca^{2+} transport rate, which warrants further investigations. Furthermore, future studies should include the molecular determinants regulating TRPV5 activity (Table 1) as targets for novel therapeutics in Ca^{2+} homeostasis-related disorders.

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